The Beneficial Effects of Probiotics in Grade II Dengue Hemorrhagic Fever

Grace L.Uy, MD[*], Felizardo N. Gatcheco, MD[#], Luisito L. Ruiz, MD[^]

*Philippine Children's Medical Center, #Jose Reyes Memorial Medical Center,

^Muntinlupa Medical Center

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ABSTRACT:

Dengue Hemorrhagic Fever (DHF) is an emerging infection in the tropical countries. It can cause hemorrhage and capillary permeability which can lead to shock and death. This could be due to T-cell activation leading to cascade of cytokines leading to disregulation of type I response which is pro-inflammatory. Probiotics have been known to modulate immune responses which could down regulate the pro-inflammatory cells. 36 patients with DHF Grade II were randomized into control and probiotics group. Probiotics were given for 7 days or until hospital discharge. Results revealed that temperature, hematocrit and platelets normalized earlier by about 2, 1.7 and 2.2 days respectively. There were no adverse events noted. Therefore, probiotics in DHF Grade II could shorten the course of the disease without causing any untoward consequences.

Dengue Fever (DF) has become an emerging infection affecting the tropical countries. It is a mosquito-borne viral infection transmitted by its vector, *Aedes aegypti.* The dengue virus is a zoonotic flavovirus with four serotypes, DEN 1,2,3,4. it has frequent outbreaks occurring in 3-4 cycles. Currently, 100 million cases of DF and .100,000 cases of Dengue Hemorrhagic Fever (DHF) occur each year.

DF is generally asymptomatic or a mild, undifferentiated fever with diphasic form. The critical stage is reached at defervescence, where hemorrhage and capillary permeability occurs classifying this now as dengue hemorrhagic fever. This is manifested by spontaneous hemorrhage or a positive tourniquet test with >9 petechiae/2.5 m^2, thrombocytopenia (<100,000/mm^3) and capillary leakage (increase in hematocrit >20% or serous effusion). Capillary leak resolves in 48 hours in uncomplicated cases, but it can cause shock and death if without appropriate supportive measures.

Virus replication in macrophage by heterotypic antibodies supposedly contributes to the development of DHF. Due to the augmented viral replication, viral antigen production and

enhanced stimulation of cellular immunity with subsequent release of cytokines and complement leads to DHF. The rapid release of cytokines caused by activation of T cells and by lysis of infected monocytes mediated by cytotoxic lmphocytes result in the plasma leakage and hemorrhage that occur in DHF. Pathology of fatal cases shows an increased activity of B-lymphocyte system, with active proliferation of plasma cells and lymphoblastoid cells, and active germinal centers.[1] The main mechanism for development of DHF is not fully known, but one contributory factor is enhancement of virus replication in macrophages by heterotypic antibodies. This anti-body dependent enhancement could result in an amplified cascade of cytokines and complement activation, causing endolethial dysfunction, platelet destruction, and consumption of coagulation factors leading to plasma leakage and hemorrhagic manifestations [2,3,4,5,6]. There is also strong evidence of T-cell activation in vivo and such activation of CD4+ and CD8+ T cells is greater in patients with DHF than those with milder dengue fever. The cytokine cascade targeting vascular endothelial cells is primarily responsible for the critical pathologic event of creating an endothelial "sieve" effect leading to fluid and protein leakage.[7] This cascade if events generated due to disregulation of type 1 response which is pro-inflammatory, leading to increased levels of TNF-α and IFN- γ.[8]

Probiotics may be defined as viable microorganisms which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition.[9] Microorganisms that are commonly regarded as human probiotics are represented by the following genera: Lactobacillus, Streptococcus and Bifidobacterium, but Enterococci and yeasts have been used.[10,11]

Some of the mechanisms of actions of probiotics are (1) abtagibusn if pathogens directly through production of antimicrobial and antibacterial compounds such as cytokines and butyric acids; (2) competer for binding and receptor sites that pathogens occupy; (3) improve function and stimulate immunomodulatory cells; (4) compete with pathogens for available nutrients and other growth factors. Probiotucs can exert positive effects without eliciting a harmful response.[12,13]

It is hypothesized that probiotics can downregulate pro-inflammatory cells which can halt the progression of plasma leakage and hemorrhage in DHF.

GENERAL OBJECTIVE:

To assess the clinical efficacy of oral intake of OMX probiotics capsule in pediatric patients with DHF Grade II.

SPECIFIC OBJECTIVES:

1. To describe the demographics of pediatric patients admitted with DHF Grade II
2. To determine whether a difference in platelet count exists between patients given OMX probiotics capsule and control patients
3. To determine whether a difference in hematocrit exists between patients given OMX probiotics capsule and control patients
4. To determine the length of time for normalization of platelet count and hematocrit between patients given OMX probiotics capsule and control patients
5. To note related adverse events such as diarrhea, vomiting

METHODOLOGY:

Research Design: Prospective, randomized controlled trial, open label

Enrollment center: Philippine Children's Medical Center

Subjects' inclusion criteria:

1. aged 6 months to 18 years of age
2. diagnosis of DHF Grade II fulfilling World Health Organization criteria (Appendix I)
3. capacity to take oral medications

Exclusion criteria:

1. known hypersensitivity reaction to probiotics
2. presence of comorbidities (e.g. pneumonia, diarrhea, etc.) at time of admission
3. presence of severe malnutrition as defined by Waterlow classification
4. presence of shock as defined by World Health Organization
5. concurrent intake of other medications not indicated for standard care of DHF

PROCEDURE

Patients' age, sex were noted. Using table of random numbers, they were divided into control and experimental group. Eligible subjects in the experimental group were given OMX Probiotics capsules 1 capsule twice a day per orem. OMX Probiotics capsule contains the following organisms: Lactobacillus bulgaricus, L. casei subsp rhamnosus, L. fermentum, L. plantarum, Bifidobacteria bifidus, B. breve, B infantis, B longum, B lactis. The capsule can be swallowed whole or contents squeezed and swalled, whichever is more convenient for the patient.

Upon enrollment, patients' vital signs which included temperature, blood pressure were noted and also recorded everyday. Baseline laboratories – hematocrit, platelets will be done everyday until discharge. Tourniquet test was done on initial examination, (midpoint of patients's blood pressure – systole + diastole/2 for 3 minutes, positive is >20 petechiae per square inch). Patients who already have full-blown petechiae were exempted from this test.

The OMX probiotics capsule were given for 7 days or until hospital discharge, whichever came earlier. The OMX Probiotics capsules were withdrawn upon onset of shock or adverse events. Subjects were monitored during their hospital stay for outcome measures stated below. The assessors of the outcome parameters were blinded as to the group to which the patients were randomized. Likewise, the laboratory personnel were also unaware of the grouping of the patients.

Discharge criteria included clinical stability, absence of fever, continuing drop of hematocrit equal to or greater than 20% baseline, and normalizing (increasing trend) of platelet count.

Shock was described as the presence of DHF with evidence of circulatory failure manifested by rapid and weak pulse, and narrow pulse pressure (<20mm Hg [2.7kPa]). It can also be manifested by hypotension for age and cold, clammy skin and restlessness.

1. clinical signs
    a. temperature
    b. blood pressure
    c. hemorrhagic tendencies as evidenced by at least one of the following:

c1. Positive tourniquet test

c2. petechiae, ecchymoses, purpura

c3. Bleeding from mucosa, gastrointestinal tract, injection sites

2. laboratory parameters
    a. hematocrit
    b. platelet count

3. Adverse events were assessed according to period of onset and duration, extent of causation (definitely related, probably related, possibly related, possibly not related, probably not related, definitely not related) and degree of severity whether nonserious or serious, and whether mild, moderate or severe. Adverse events monitored included recurrence of temperature, development of diarrhea, hypersensitivity reactions such as rashes, pneumonia, liver dysfunction, continuous bleeding, and sepsis.

   Treatment failure was defined as patients who developed shock or who developed adverse conditions such as defined above. Patients who withdrew from the study with at least 2 days participation were included in the analysis.

   For statistical analysis, T-test was used to compare means of outcomes between the two groups. P value <0.05 was considered significant.

SAMPLE SIZE DETERMINATION

$$n = \frac{Z^2 (2\gamma)^2}{d^2}$$

Where:

n = desired sample size
γ = the standard deviation of the parameter of interest, i.e., difference between the two means
Z = the normal deviate corresponding to the reliability level desired for the estimate
d = the maximum permissible error for the difference between two means

Using Hematocrit

$$n = \frac{(1.96)^2\,(2[.0216025])^2}{(.02)2}$$

$= 17.92751813 \sim 18$ per group

Using Platelet

$$n = \frac{(1.96)^2\,(2[80.9936])^2}{(100)^2}$$

$= 10.080974 \sim 11$ per group

RESULTS

36 patients were enrolled in the study with 18 patients in the experimental and control groups, respectively. All patients were able to tolerate the OMX probiotics capsule without any problems.

Comparing the mean age of subjects on admission between the two groups, the results showed that there was o significant difference noted as proven by the p value of 0.34. Moreover, there was no significant difference in the proportion of males and females (p=0.17) (Table1).

Table 1. Comparison of Age and Sex Between the Two groups

	Groups		**P value**
	Control (n=18)	**Experimental** (n=18)	
Age (in mos)			
Mean +/- SD	124.33 +/- 56.38	107.06 +/- 52.34	0.34 (NS) (t-test)
Range	31 -200	32 – 219	
Sex			
Male	13 (72.2%)	9 (50.0%)	0.17 (NS)
Female	5 (27.8%)	9 (50.0%)	(chi-square test)

There was significant difference in the average temperature, mean difference in the temperature on admission and upon discharge and the day normalization of temperature was achieved as shown by p values 0.04, 0.001 and 0.007 respectively. However, it is noted that the temperature range falls within normal limits and is not in anyway a reflection of pathology. (Table 2).

Table 2. Comparison of the Average Temperature, Difference in Temperature and Day Normalization in Temperature was achieved between the Two Groups

	Groups		P value
	Control (n=18)	**Experimental** (n=18)	
Average Temp.			
Mean +/- SD	37.24 +/- 0.52	36.91 +/- 0.42	0.04 (S)
Range	36 – 38.3	36.1 – 37.5	(t-test)
Diff. in Temp.			
Mean +/- SD	1.21 +/- 0.92	0.32 +/- 0.63	0.01 (S)
			(t-test)
Day Normalized			
Mean +/- SD	2.50 +/- 3.01	0.50 +/- 1.20	0.007 (S)
Range	0 – 11	0 – 4	(t-test)
Median	2	0	

Negative value indicates increase in values upon discharge

Blood pressure is broken down into systolic and diastolic components. Within each groups, comparing the initial and the systolic BP of subjects upon discharge, there was a significant difference noted in the experimental group only with p value = 0.005. There was a significant increase in the systolic BP of subjects in the experimental group (Table 3). Within each groups, comparing the initial and the diastolic BP of subjects upon discharge, there was a marginally significant difference noted in the experimental group with p value = 0.09. There was a marginally significant increase in the diastolic BP of subjects in the experimental group (Table 4).

Table 3. Comparison of Initial and Upon Discharge Systolic BP of Subjects Between the Two Groups

Systolic BP	Groups		P value
	Control (n=18)	**Experimental** (n=18)	
Initial			
Mean +/- SD	102.78 +/- 11.28	93.89 +/- 9.16	0.01 (S) (t-test)
Range	90 – 12	80 – 110	
Upon Discharge			
Mean +/- SD	101.94 +/- 9.26	100.89 +/- 12.37	0.77 (NS) (t-test)
Range	90 - 120	90 – 130	
P Value	0.70 (NS)	0.005 (S)	

Table 4. Comparison of Initial and Upon Discharge Diastolic BP of Subjects Between the Two Groups

Diastolic BP	**Groups**		**P value**
	Control (n=18)	**Experimental** (n=18)	
Initial			
Mean +/- SD	68.33 +/- 8.58	62.78 +/- 8.26	0.06 (NS) (t-test)
Range	60 – 80	50 – 80	
Upon Discharge			
Mean +/- SD	68.33 +/- 6.18	65.56 +/- 7.84	0.24 (NS) (t-test)
Range	60 - 80	60 – 80	
P Value	1.00 (NS)	0.09 (NS)	

Table 5. Comparison of Initial and Upon Discharge HCT of Subjects Between the Two Groups

HCT	**Groups**		**P value**
	Control (n=18)	**Experimental** (n=18)	
Initial			
Mean +/- SD	0.421 +/- 0.059	62.78 +/- 8.26	0.06 (NS) (t-test)
Range	0.350 – 0.540	50 – 80	
Upon Discharge			
Mean +/- SD	68.33 +/- 6.18	65.56 +/- 7.84	0.24 (NS) (t-test)
Range	60 - 80	60 – 80	
P Value	1.00 (NS)	0.09 (NS)	

Table 5.1. Comparison of Average HCT, Difference in HCT and Day Normalization in HCT was Achieved Between the Two Groups

	Groups		**P value**
	Control (n=18)	**Experimental** (n=18)	
Average HCT			
Mean +/- SD	0.405 +/- 0.037	0.379 +/- 0.045	0.06 (NS)
Range	0.355 – 0.486	0.285 – 0.447	(t-test)
Diff in HCT			
Mean +/- SD	0.045 +/- 0.039	0.043 +/- 0.056	0.90 (NS) (t-test)
Day Normalized			
Mean +/- SD	+/- 2.98	2.16 +/- 1.88	0.04 (S)
Range	0 - 10	0 – 6	(t-test)

Negative value indicates increase in values upon discharge

Within each groups, comparing the initial and the HCT of subjets upon discharge, there was a significant difference noted in the control and experimental group with p values <0.001 and 0.004 respectively (Table 5). There was a marginally significant difference in the average HCT of subjects between the two groups (p=0.06). Comparing the mean day normalization of the HCT was achieved between the two groups, there was a significant difference noted as proven by the p value of 0.04. The mean day for the experimental group to have normal HCT was significantly shorter than the control group (p=0.04) with a mean of 3.94 and 2.16 days for the control and experimental groups respectively (Table 5.1).

Table 6. Comparison of Initial and Upon Discharge Platelet Count of Subjects Between the Two Groups

Platelet Count	**Groups**		**P value**
	Control (n=18)	**Experimental** (n=18)	
Initial Mean +/- SD Range	103.83 +/- 26.33 36 – 139	106.72 +/- 50.66 35 – 222	0.83 (NS) (t-test)
Upon Discharge Mean +/- SD Range	213.11 +/- 65.37 104 – 369	215.94 +/- 83.62 125 – 423	0.91 (NS) (t-test)
P Value	<0.0001 (S)	<0.0001 (S)	

Table 6.1. Comparison of Average Platelet Count, Difference in Platelet Count and Day Normalization in Platelet Count was Achieved Between the Two Groups

	Groups		**P value**
	Control (n=18)	**Experimental** (n=18)	
Average Pltlt Ct. Mean +/- SD Range	126.88 +/- 25.27 90 – 170	142.5 +/- 35.01 70 – 210	0.13 (NS) (t-test)
Diff in Pltlt Ct. Mean +/- SD	-109.28 +/- 66.81	-109.22 +/- 96.31	0.99 (NS) (t-test)
Day Normalized Mean +/- SD Range	5.50 +/- 2.22 3 – 11	3.33 +/- 2.68 0 – 10	0.01 (S) (t-test)

Negative value indicates increase in values upon discharge

Within each groups, comparing the initial and the platelet count of subjects upon discharge, there was a significant difference noted in the control and experimental group with p values <0.0001 for both (Table 6). There was no significant difference in the average platelet count and the difference in platelet count fro initial until upon discharge of subjects between the two groups as shown by the p values 0.13 and .99 respectively. However, there was a significant difference noted in the mean day of normalization as proven by the p value of 0.01. The mean day for the experimental group to have normal platelet count was significantly shorter than the control group (p=0.01) with a mean of 5.50 and 3.33 days for the control and experimental groups respectively (Table 6.1).

DISCUSSION

DHF continues to be one of the feared diseases because it could have fatal consequences. It presentation could range from simple flu-like illness to overt shock. There is no treatment being offered nowadays, but only simple supportive measures such as fluids and blood products. We were able to present in this paper that patients with DHF Grade II could be reversed earlier when given probiotics.

Review of literature did not yield any research or study on the use of probiotics in DHF. This is the first study undertaken to demonstrate whether probiotics would have any effects in this disease.

This study has shown that patients with DHF Grade II given OMX probiotics capsule did not progress to further deterioration of their condition and were able to have earlier normalization of their hematocrit and platelet count.

The temperature also was noted to normalize earlier in the OMX probiotics capsule group by about 2 days. This positive change could also lead to better clinical picture and signal slowing down of the inflammatory process.

As to the effect on blood pressure, there was no significant effect clinically in both groups. Although blood pressure is an important clinical tool to assess the clinical stability of patients, it is not affected by the use of probiotics.

Hem concentration as manifested by elevated hematocrit is a manifestation of loss of plasma from the vascular compartment. Thrombocytopenia is a reflection of disorder in homeostasis. They are probably due to the proliferation and release of proinflammatory cytokines such as interferon gamma and tumor necrosis factor alpha.[8]

In patients who present with DHF, the most feared sequelae would be further plasma leakage and thrombocytopenia, which could lead to shock and death. Probiotics in this study have shown that it could reverse the trend by normalizing the hematocrit and increasing platelet count earlier, by 1.7 and 2.2 days respectively. Probiotics may stabilize the intestinal gut flora by halting the cytokine cascade through downregulation of the inflammatory cells and cytokines. It probably also control overgrowth of potentially pathogenic microorganisms with viral origin by antagonizing noxious or unwanted microorganisms, eliminate toxins and stimulate intestinal immune defense.

With the tendency of the hematocrit to normalize earlier the platelet by 0.5 days, this might demonstrate that this hematocrit will recover first with plugging of the vascular leaks and therefore with recovery of the vascular disorders. This could mean an interplay between the two factors with one leading the other to either recovery or deterioration.

When patients recover earlier, by as much as two days, it would mean an earlier return of the patients' clinical well-being, with improvement in appetite, faster discharge from medical care and therefore lesser use of resources.

We could therefore infer from this study that patients with Grade II DHF could benefit from probiotics early on their disease and that further progress to Grade 3 or 4 DHF could be prevented. Furthermore, it was tolerated by the patients and did not demonstrate any ill-side effects. With regard the other parameters (like BP) wherein no statistical significance were noted, this might be the effect of the limited sample size and would therefore warrant further investigation.

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